(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

CORRECTED VERSION

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 June 2000 (22.06.2000)

PCT

(10) International Publication Number
WO 00/36093 A3

(51) International Patent Classification[7]: C12N 9/00, 9/14, C07K 1/04, G01N 33/50, C12N 15/62, 15/10

(21) International Application Number: PCT/US99/30162

(22) International Filing Date:
18 December 1999 (18.12.1999)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/112,723	18 December 1998 (18.12.1998)	US
60/158,077	7 October 1999 (07.10.1999)	US

(71) Applicant *(for all designated States except US)*: **THE PENN STATE RESEARCH FOUNDATION** [US/US]; 304 Old Main, University Park, PA 16802 (US).

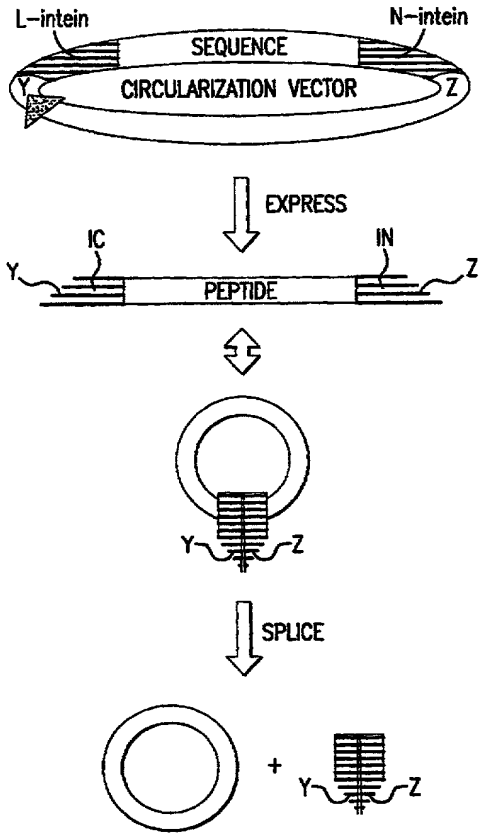
(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **BENKOVIC, Stephen, J.** [US/US]; 771 Teaberry Lane, State College, PA 16801 (US). **SCOTT, Charles, P.** [US/US]; 1614 Highlandon Court, State College, PA 16801 (US). **ABEL-SANTOS, Ernesto, V.** [US/US]; 1635 Highlandon Court, State College, PA 16801 (US).

(74) Agents: **MONAHAN, Thomas, J.** et al.; The Pennsylvania State University, 113 Technology Office, University Park, PA 16802-7000 (US).

[Continued on next page]

(54) Title: INTEIN-MEDIATED CYCLIZATION OF PEPTIDES



(57) Abstract: Methods of producing cyclic peptides and splicing intermediates of peptides in a looped conformation are disclosed. The methods utilize the trans-splicing ability of split inteins to catalyze cyclization of peptides from a precursor peptide having a target peptide interposed between two portions of a split intein. The interaction of the two portions of the split intein creates a catalytically-active intein and also forces the target peptide into a loop configuration that stabilizes the ester isomer of the amino acid at the junction between one of the intein portions and the target peptide. A heteroatom from the other intein portion then reacts with the ester to form a cyclic ester intermediate. The active intein catalyzes the formation of an aminosuccinimide that liberates a cyclized form of the target peptide, which spontaneously rearranges to form the thermodynamically favored backbone cyclic peptide product. Also disclosed are nucleic acid molecules, polypeptides, methods for making cyclic peptides, methods of making libraries, and methods of screening peptides.

WO 00/36093 A3